Antibody Tests for CoVID-19: Essential information for decision makers

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INTRODUCTION

There are two means for definitive testing for CoVID-19 disease:

1. Test for antibodies in blood
2. RT-qPCR Test for the virus antigen by identifying its exact genetic code (RNA) in the sample.

Each approach is complementary, with their own benefits and limitations. This paper discusses what these are, with a focus on antibody testing.

Deep Life Medical declares that it supplies both types of test. In so doing, it claims to be neutral in the presentation of their relative advantages and limits.

We cover crucial information regarding the accuracy and limits of antibody field tests, and answer frequently asked questions on what the field test kits can and cannot do.

The virus that causes the CoVID-19 disease is SARS-CoV-2. When referring to the virus in this paper the viral name is used, when referring to the disease, CoVID-19 is used.

How does CoVID-19 compare to the Flu?

There are two factors to consider: how infectious the SARS-CoV-2 virus is, and how deadly.

The SARS-CoV-2 virus is highly infectious, with a rate of replication above 1.3 daily. If control measures to limit spread are not taken, between 70% and 80% of the population will be infected: this figure can be calculated directly from the replication rate of the virus.

The mortality rate for CoVID-19 is between 0.4% and 12% of those infected. The top end figure of 12% mortality is measured from the population of those diagnosed in hospital. Many people will have milder symptoms and not be included in this count because they do not present at hospitals. Alternative estimates based on the excess deaths recorded in Q1 2020 compared to the same months in 2019, indicate around 4% mortality. Antibody tests in the general population are reporting more people infected, therefore a still lower mortality, down to 0.4%. As of April 2020 nobody knows the true figure for CoVID-19 mortality, but a figure of 4% is realistic, and anywhere in this range from 0.4% to 12% is a highly dangerous disease.

In addition to the mortality figure, a similar proportion of the population will suffer long term damage from the disease.

The combination of high infection rate and high mortality or long term damage is what makes CoVID-19 a very serious threat.

Comparing with the H1N1 flu, SARS-CoV-19 is 1000 times more infectious and the mortality rate of 4% is 40 times that of recent H1N1 epidemics. This makes CoVID-19, a threat that is 40,000 times more serious than H1N1 flu.

Every pandemic starts slowly, which can delay response that prevents spread. If cases double every 3 days, a single case becomes 100,000,000 after 80 days (2½ months). However, a month into the crisis, there are under 1000 cases. It is crucial to stop the spread while the numbers infected are small.

Deep Life Medical can provide copies of the scientific studies and reports behind these numbers.

How can tests help reduce the impact?

Control measures reduce the proportion of the population who will be infected. For the reasons described above, measures taken early are vastly more effective than measures taken late.

Australia has been successful in controlling CoVID-19. It took action early. As of end April 2020, less than 7,000 were infected. This is under 0.03% of Australia's 25 million population.

Effective control measures involve a package of actions, including:

1. detecting and isolating infected individuals and tracing their contacts
2. hand washing frequently and more thoroughly
3. social distancing: avoiding non-essential interaction and keeping apart from others
4. wearing facemasks without one-way valves, limiting the distance expired droplets travel
5. stopping new spreaders arriving from overseas or outside the infected territory
6. vaccines when available, or medication to reduce the infective period if no vaccine.

If infected persons cannot be identified and contacts traced, then entire communities are locked down, with a social and economic impact that can be on a similar scale to the disease.

Testing is key to identifying infectious individuals to avoid lockdowns, or to remove lockdowns, and to protect critical infrastructure. Testing is also critical in many other aspects of managing the crisis. Deep Life Medical offer a White Paper called “Testing: the key to managing the CoVID-19 pandemic” describing these aspects.

How to Diagnose COVID-19?

The accurate and timely diagnosis of SARS-CoV-2 infection is critical in the effort to provide appropriate treatment for patients, to limit further spread of the virus and ultimately, to eliminate the disease from human society.

Symptoms on their own are not a positive diagnosis for CoVID-19, as they are not easily distinguishable from pneumonia induced by infection with other common respiratory tract pathogens. Moreover, the severity of symptoms does not indicate the severity of COVID-19 illness. Patients with mild outward symptoms can succumb over the course of minutes.

Definitive diagnosis of COVID-19 involves:

1. A positive test result from a CoVID-19 specific test.
2. Symptoms associated with COVID-19, as published and updated by the WHO or the national Health Authority.

A positive test on its own does not confirm a diagnosis, as the person may have been infected previously. Either a positive test result and symptoms must be present, or both antibodies and the viral RNA tests must be positive.

If a person is showing symptoms and has tested negative, then it should be double checked using an alternative type of test: RT-qPCR to test for the viral RNA if an antibody test was negative, or an antibody test if RT-qPCR RNA testing was negative.

Combining RT-qPCR and antibody detection significantly improves the sensitivity of pathogenic diagnosis for COVID-19.

If no antibody test is available, nasal-pharyngeal swabs can be used with RT-qPCR to test for the viral RNA directly. If this is done, care must be taken to avoid environmental contamination. A RT-qPCR can pick up even single virions which may be on nasal hair or mucus if an individual has been in the vicinity of someone infected, without the individual being infected himself. This will create a false positive RT-qPCR RNA result. If RT-qPCR alone gives a positive result, it is recommended to repeat the test to cover a different form of sample, such as nasal-pharyngeal swabs and mucosal aspirate, or check using an antibody test.

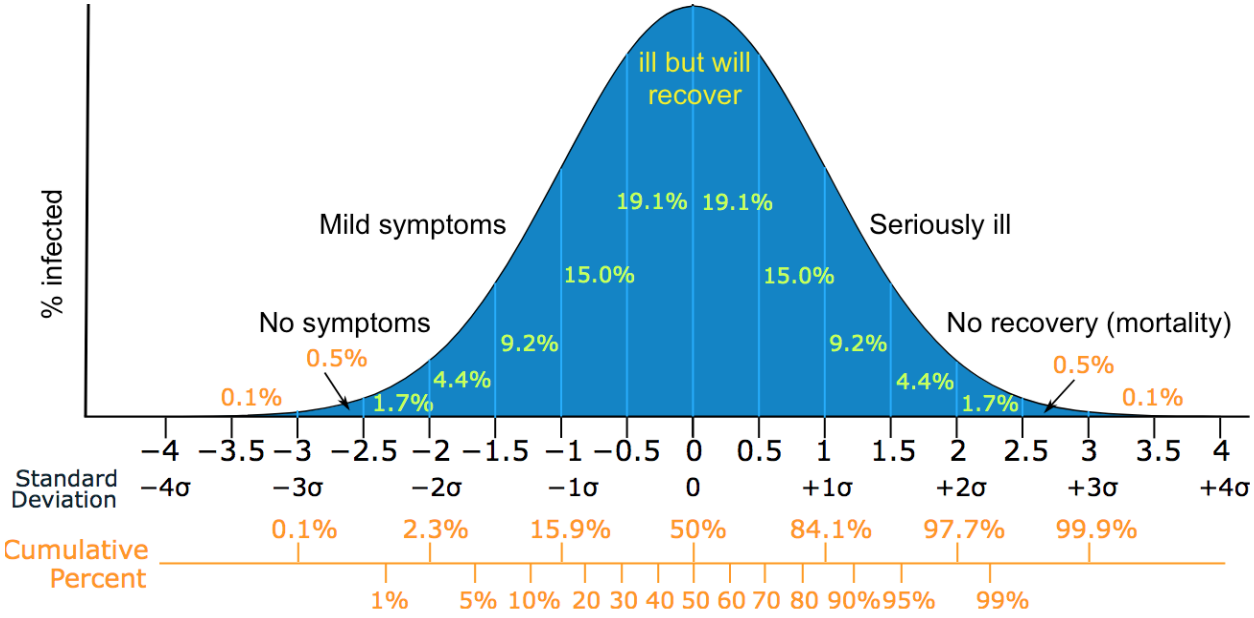
False Negatives in Antibody Testing

There is a wide variation in how different people fight off disease.

A person may be infected and spreading SARS-CoV-2, without them knowing it. Only later will they display CoVID-19 symptoms. Some people will recover without even noticing any symptoms. Studies indicate 20% of people may fall into this category of never showing any symptoms – these are mostly children and young people.

At the other end of the spectrum are 20% of people who suffer serious respiratory distress. Some of these will die. These are mostly older people, but those of all ages can succumb to CoVID-19.

In between these two extremes, are the bulk of the population who suffer symptoms, feel terrible, but recover without needing specialist treatment.



There is a similar variation in how rapidly a person develops detectable antibodies. Antibody tests will show a negative until there are enough antibodies in the blood to be detectable.

Antibody tests rarely produce a false positive result: if the result is positive then they are either currently infected with a coronavirus or have been infected and recovered. The attention is on false negatives because of the variation in time different individuals take to produce antibodies to the infection.

From the moment of infection it generally takes 3 to 7 days for antibodies to be detectable. Most people will show antibodies by the time symptoms appear. Antibodies will continue to rise until 8 to 15 days after infection starts. The clinician should be aware that some people may never show antibodies.

It can be seen from this that the antibody test is a first line of testing and screening. It is a tool used in conjunction with RT-qPCR testing for the viral RNA, to obtain solid data on who is infected, how many are infected, how well their body is fighting the infection and the numbers who have recovered.

When to use RT-qPCR and When to use Antibody tests?

Most countries choose to run both types of test: antibody assay tests and RT-qPCR tests. Each have their role. They complement each other, lead to definitive diagnosis and tracking of progress.

Deep Life Medical's CoVID-19 antibody field tests are:

1. Fast, taking just 10 minutes from blood sample to result, compared to more than an hour for RT-qPCR nasal-pharyngeal swabs.
2. Accurate: independently validated to be within 98% of the accuracy of RT-qPCR in detecting antibodies.
3. Cheap: antibody tests are a quarter of the cost of RT-qPCR.
4. Low work load taking under a minute to take a sample, start the test, then after 10 minutes, to read the result.
5. Comfortable: taking a pin-prick blood sample from the patient using the safe lancets is more comfortable for patients than a deep nasal-pharyngeal swab needed for RT-qPCR for the viral RNA.

With these advantages of antibody tests, why choose RT-qPCR? The reason is antibody kits have two limitations, namely:

1. As described already, the time lag between infection and showing detectable antibodies is typically 3 to 7 days, but it can be longer in some individuals. Antibodies continue to rise until between 8 and 15 days after the infection started, but some individuals do not show detectable antibodies.
2. A second limitation of antibody tests is that some patients who have had a coronavirus virus, but since recovered, may show positive for a period of time, giving a false positive. To avoid this, the positive test is considered in conjunction with observable symptoms before reaching a diagnosis.

The RT-qPCR viral RNA test determines conclusively whether the patient is currently infected, without either of these drawbacks. The drawbacks of RT-qPCR are:

1. RT-qPCR is requires a laboratory, is a high workload and is slow: that limits throughout. The Deep Life RT-qPCR kits address that issue by greatly improving RT-qPCR test safety and throughput, however there is still more than an hour between a patient being presented and results being available.
2. RT-qPCR is so sensitive it can result in false positives due to contamination in the environment. Our mucosal tissue is so good at filtering particles from inhaled air that a person who has been in the proximity of someone infected, may deposit sufficient virions into a nasal swab to show a positive, when the individual is not infected.
3. RT-qPCR is around four times the cost of an antibody test.

For these reasons:

- Antibody testing is more suitable for community testing, triage or in field hospitals.
- RT-qPCR is suited to testing in well equipped hospitals, and for screening blood in blood banks, with antibody testing performing the double-check.

Both types of test have a role in limiting the spread of disease. Used together they increase significantly the accuracy of diagnosis and management of the disease.

What do Antibody Tests look like, and what is the test process?

Lateral flow antibody tests are biochemical reagents printed on a paper strip. The strip is presented in one of two ways:

1. Bare strips in a canister,
2. Cassettes such as that shown in the image on the right. A cassette is a white plastic moulding around the strip, that makes it easier to place the blood sample, buffer and read the results.
   Each test is in a date coded sachet with silica gel. Each pack is supplied with pipettes and instructions. Lancets are usually purchased with the kits. Alcohol wipes and basic PPE are also required.

The antibody test process is simple:

1. Patient offers a finger tip, which the clinician cleans with an alcohol wipe.
2. Unistik 3 Lancets provide a painless means to take the blood sample. The grey lancet cap is removed by turning it, the end is placed on the side of the finger tip and the lancet button is pressed. The lancet tip shoots out and back in again, eliminating risk of exposed sharps. A drop of blood then forms on the skin.
3. Wipe away the first blood, then take a sample of a large second droplet using the pipette supplied with the kit.
4. Deposit the blood on the S position of the test strip or cassette, laid on a flat surface.
5. Put two drops of the buffer in the B position.
6. Wait 10 minutes and read the result. The reading becomes invalid after 20 minutes.

Above: A box of lancets, timer and the CoVID-19 test in a cassette, with buffer solution alongside. After 10 minutes the results are visible: in this case the patient is infected as the control line is visible (C), and both the IgG and IgM lines are visible.

Reading the test is just a matter of observing the lines on the strip after 10 minutes:

- If a control line and either or both the IgG and IgM test lines are seen, the sample is positive.
- If only a control line is seen, the result is negative.
- If no control line is seen, the test is void and should be repeated.

Deep Life Medical offer a 5 minute training video for the clinician to perform this procedure. If required, Deep Life Medical can also provide accredited instructor training over Skype or Zoom, or in country.

How Lateral Flow Antibody Tests work

CoVID-19 lateral flow tests detect the glycoproteins on antibodies that are produced in response by the body to the SARS-CoV-2 virus. The test is specific for immune glycoproteins produced by coronavirus antibodies. These glycoproteins are called Immunoglobulin G and M (IgG and IgM).

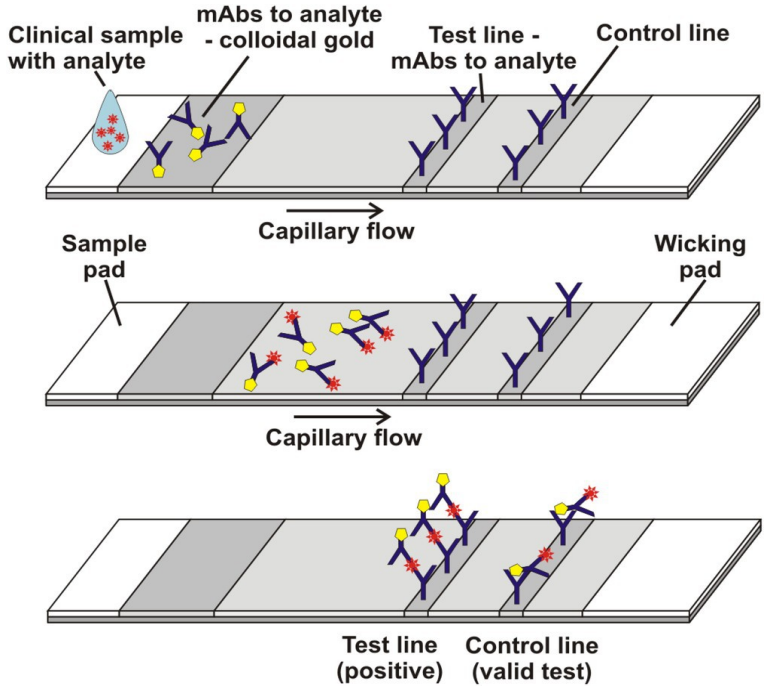
Immunoglobulins are normally produced by the immune system in response to bacterial or viral infections and act to 'flag' the pathogen, so that other immune cells can detect and kill it. The IgG/IgM that the test detects are specific to the SARS-CoV-2 virus, so a positive result would indicate the patient has contracted CoVID-19 disease and not some other infection.

Since there are two analytes of interest (IgM and IgG), there are two test lines on the strip. Only one test line needs to be seen for a positive diagnosis (as well as the control line). Two test lines would also indicate a positive result.

The sample (blood, saliva etc.) is added to one end of the test strip, along with a buffer solution. The sample flows along the test strip via capillary action into a reservoir of antibodies that are specific to the analyte of interest. If the analyte is present in the sample, the antibodies in the blood sample will bind to it and continue to flow along the test strip. It will then reach a test line, which contains a second set of bound monoclonal antibodies (mAbs) specific to the analyte. The analyte will bind to these (if present) and will be immobilised along the line. The remaining sample will continue to flow until it reaches a final line, known as the control line. The control line contains more bound antibodies, but these are specific to the flowing antibodies themselves, not the analyte. The remaining antibodies are captured on the control line.

The first section of the test strip has an area containing monoclonal antibodies with coloured dye or gold nanoparticles, so the line can be seen with the naked eye when they bind on the test lines.

Below is an info-graphic showing this lateral flow test process.



Above: Summary of the CoVID-19 antibody lateral flow test, showing movement of the analyte across the test strip

There are other types of antibody test, such as chemiluminescence immunoassays, which are closer in concept to ELISAs than lateral flow assays. These tests generate a light signal proportional to SARS-CoV-2 IgM antibodies. However the basic concept of binding a monoclonal antibody to the gylcoproteins in the analyte remain.

How accurate are Deep Life Medical's Antibody tests?

The antibody tests offered by Deep Life Medical were validated by independent laboratories by comparison with a leading commercial PCR for the same antibodies in the same set of 181 samples.

IgG Results

Method	CoVID-19 IgG test line	PCR Proven Positive Samples	PCR Proven Negative Samples	Total Results	% True	% False
Sensitivity	Test shows IgG Positive	37	1	38	97.37	2.63
Specificity	Test shows IgG Negative	1	142	143	99.30	0.70
Accuracy	Total	38	143	181	98.88	1.11

- Sensitivity: 97.4% (95% Confidence Interval: 86.2%-99.9%)
- Specificity: 99.3% (95% Confidence Interval: 96.2%-99.9%)
- Accuracy: 98.9 % (95% Confidence Interval: 96.1%-99.9%)

IgM Results

Method	CoVID-19 IgM test line	PCR Proven Positive Samples	PCR Proven Negative Samples	Total Results	% True	% False
Sensitivity	Test shows IgM Positive	33	2	35	94.28	5.71
Specificity	Test shows IgM Negative	5	141	146	96.57	3.42
Accuracy	Total	38	143	181	95.43	4.57

- Sensitivity: 94.28% (95% Confidence Interval: 71.9%-95.6%)
- Specificity: 96.57% (95% Confidence Interval: 95.0%-99.8%)
- Accuracy: 95.43% (95% Confidence Interval: 92.2%-98.4%)

By using two independent test lines, the accuracy is combined for a 99.994% accuracy when both lines are present.

This validation process is repeated frequently as part of the acceptance process into different countries.

How Accuracy is Measured

RT-qPCR is the reference standard for assessing tests.

The accuracy of antibody test kits is determined by measuring a sample against RT-qPCR.

There are two ways to do this:

1. Use RT-qPCR to confirm the antibodies are in the patient's blood and use that same blood sample for the test of the lateral flow antibody kit.
2. Use RT-qPCR to test for the RNA of the virus on a nasal-pharyngeal swab, then assume the patient is infected and assume the patient is developing antibodies.

The first approach is solid science and is how the quoted results were obtained.

The second approach requires care because the RT-qPCR RNA test is not looking for the same thing as the antibody test. In particular:

1. Environment false positives in RT-qPCR. If a nasal-pharyngeal sample is taken from an individual who has been in the vicinity of an infected person, the swab may pick up virions in the nasal passages that are not an infection: they are simply environmental contamination trapped by the nasal mucus and hair. To avoid this error when comparing kits, it is important only to take RT-qPCR samples from those showing clinical symptoms. One study found that in Wuhan 46% of field RT-qPCR samples were contaminated: hence antibody tests are preferred for this field role.
2. Whilst RT-qPCR can detect a single RNA strand from a virion shortly after the moment of infection, antibody kits require some days for an antibody response from someone infected. In this time gap, the RT-qPCR RNA tests can show positive, when the antibody test shows negative. To avoid this error when comparing tests, it is important only to take RT-qPCR samples from those showing clinical symptoms.
3. As the body responds to infections, the IgM levels drop, so if the comparison patients are into the recovery phase, then the IgM line may not be present though the IgG line will be. This is why the antibody kits have two lines for the IgG and IgM immune glycoproteins.

RT-qPCR is not perfect: there is a small degree of uncertainty for results, as the process involves setting thresholds as well as it comprises many stages, each of which can introduce errors. In addition the sample size used in the tests gives rise to an uncertainty in the result. These uncertainty figures are combined and presented as a confidence interval.

For example, IgG Specificity (how well a test avoids false positives) on the Deep Life Medical antibody kits is 99.3% with a 95% Confidence interval of 96.2%-99.9%. This means there is a 19 out of 20 probability that the antibody kit is in the range from perfect (99.9%) to 96.2% specific, and a 1 in 20 chance that it it could be less than 96.2%.

Following validation, the antibody test complements RT-qPCR RNA testing and can be used together for definitive diagnosis.

How Specific is the Antibody Test to CoVID-19?

The COVID-19 IgG/IgM tests offered by Deep Life Medical for whole blood, Serum and Plasma have been tested for anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV and anti-HCV positive specimens. The results showed no cross-reactivity.

Other human coronaviruses may cause a cross-reactivity but there is insufficient data to determine how much. The incidence of these earlier human coronaviruses in the general population is estimated at 0.8% based on several studies. Some regions will have a higher figure and others a lower.

Can tests detect acquired immunity?

Governments hope for tests that determine whether a person has had CoVID-19 disease, so they can determine when to lift lockdowns. No test can do that currently with 100% efficiency, nor is likely to be able to do in the near future, but the cause is not lost.

Upon exposure to SARS-CoV-2, we have explained that IgM antibodies are formed, then IgG, the IgM falls off as the body starts overcoming the virus, followed by a slower fall of IgG. Most people after recovery will show positive IgG for a period, but some of the population's IgG and IgM levels have been found to drop to undetectable levels even before leaving hospital. All people will eventually show no detectable IgG as the numbers of antibodies drop to minuscule levels. The evidence so far for CoVID-19 is this takes 9 months on average.

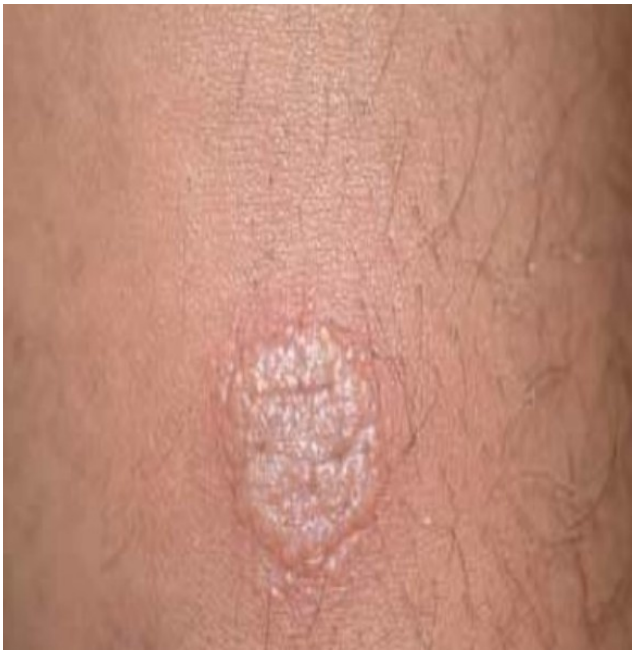
There is a great deal of variation in the rate at which Ig levels increase and decrease.

Despite this, there is a window of opportunity if samples are taken of the population repeatedly through the pandemic, to paint a reasonably accurate picture. An antibody test can detect the IgG as it is generated, and retesting can determine when it is no longer detectable for that individual. A statistical model can use this data to determine the proportion in the population who have been infected, as well as the proportion who have an antibody response who are asymptomatic. It is not necessary for the test to detect every case as this is a statistical analysis.

There is evidence that after being infected, a minority of people may be reinfected with SARS-CoV-2. For the majority, as of April 2020, it is not known how long their immunity lasts. The data of Ig levels needs to be collected now, with the expectation that the protection is lasting.

A definitive method to determine if a person has had a disease is a "test with provocation": the person is exposed to a passive version of the virus then measured a few days later to detect the response. A person who has not been previously exposed would not have a strong response, but a person who has previous exposure will have, as the body recognises the pathogen and responds faster to it.

This method is used for health screening of some bacterial and viral diseases. In some countries, prior exposure to sexually transmitted disease can be tested by couples planning to get married who wish to check on the virtue of their spouse to be, with the response measured using qPCR. The method is also used to test for TB exposure prior to vaccination – the Mantoux tuberculin skin test (TST). The patient is given an injection of neutered tuberculin that triggers the immune system if the person has been exposed previously, causing a wide blister. If only a small blister appears, then they are vaccinated.



Above: a blister from a Mantoux tuberculin skin test (TST). The diameter of the blister is measured to determine prior exposure to TB.

To detect securely whether a person has been exposed to SARS-CoV-2, would require their immune response to be woken up, i.e. by the "provocation" method. To develop that provocation method for the SARS-CoV-2 virus would take a significant amount of new research.

RT-qPCR is just as ineffective at measuring if someone has been exposed. It is used to detect response in the provocation protocol for some diseases, but without the provocation there will be nothing to detect. The provocation for a SARS-CoV-2 response has not been developed.

This means, test now or never obtain this important data needed to manage the pandemic.

Quality Control

The validation tests on the CoVID-19 tests supplied by Deep Life Medical were carried out in national laboratories, or ISO 17021/17025 laboratories with global IAF accreditation.

The antibody tests offered by Deep Life Medical are manufactured in the UK/Europe and are subject to strict quality control under a certified EN13485 quality system in the manufacturing plant, to ensure consistency. The datasheets disclose the identity of the manufacturing plant and the certifications.

Deep Life Medical recommend that all customers test antibody kits against RT-qPCR before committing procurement. Deep Life Medical fully support that process, making available small test batches of kits at the same price as batches of a million kits, and issue those test kits from current production stock to avoid delay.

The Wilds

There are around 100 suppliers of CoVID-19 antibody tests worldwide. Deep Life Medical has validated the performance of many of these before selecting which tests to supply, and then entering into formal agreements with those manufacturers. During this validation process, it was found that:

1. Tests from particular manufacturers had zero accuracy. These are simply fake tests.
2. Millions of antibody tests in March and April 2020 made by two companies in China had 30% accuracy.
3. Most manufacturers' tests were in the 80% to 91% accuracy range. None of these are useful.
4. Three companies had tests with better than 98% accuracy. In time more companies will achieve this standard. Deep Life Medical supply only those tests that achieve this level of performance, revalidating them often.

Chinese Tests

Two European governments purchased antibody tests from China then rejected them on discovering their performance was in the 30% category. Officials from the Chinese Government are reported to have claimed that these were from unauthorised plants. This version of events is most unlikely.

In February 2020, China introduced their CCC regulations requiring all medical device and PPE factories to be registered. This was enforced rigorously, with officials sealing plants that did not meet the unpublished criteria. Plants that were not wholly Chinese owned or products that were non-Chinese owned were denied registration, despite them holding European product certification. Production of the antibody test kits with the poor performance was through a CCC “authorised” company in China.

The CCC regulations caused many problems for European companies who could no longer purchase components of their products in China (Deep Life Medical included). This resulted in Chinese PPE and IVDs flooding the market. The truth is CCC certificates were issued without products being tested. Chinese companies leapt from from minor market share to dominance in the course of a few weeks by strangling the flow of component parts to non-Chinese manufacturers.

In another QA failure from China, the genetic codes issued in January 2020 for SARS-CoV-2 were incorrect, leading to less accurate CoVID-19 tests of all types (both RT-qPCR and lateral flow antibody tests). This was resolved only in late February when genetic codes from other regions of the world were released. Deep Life Medical identified this issue early and have never sold those incorrect tests.

The bottom line is the quality environment in China is entirely different to that in Europe, where products are tested and Type Approvals issued, products are registered with authorities with a portfolio of test evidence, and production is monitored and controlled by EN13485 or ISO9001 + Functional Safety certifications. A Chinese medical or PPE product is not easily compared to one from Europe.

Summary

CoVID-19 is causing a tsunami of human suffering. The disease has an impact tens of thousands times worse than H1N1 flu.

That tsunami can be tamed by taking adequate control measures. Control measures affect the lives of everyone and can do considerable harm in themselves if not managed well. Testing is the key tool in that management, in diagnosis and in effective treatment.

Antibody testing using the Deep Life Medical kits is fast, inexpensive, accurate and reliable. Antibody detection offers vital clinical information during the course of SARS-CoV-2 infection. Multiple clinical studies have concluded that there is strong empirical support for the routine application of serological antibody testing in the diagnosis and management of COVID-19 patients.

Deep Life Medical offer the highest quality of lateral flow antibody tests to support:

1. Field testing
2. Hospital triage
3. Hospitals and clinics treating patients without access to a full PCR laboratory
4. Within a hospital setting, combining RT-qPCR RNA tests and antibody detection tests significantly improves the sensitivity of pathogenic diagnosis for COVID-19
5. Rapid screening for securing critical infrastructure and facilities against the threat of spread of disease
6. Population screening to determine when it is safe to return to work, if the testing is carried out throughout the period of the pandemic. There is no means to obtain this information long after people have recovered.

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